Standard Operating Procedure for:

Quanti-Tray/2000 Colilert-18 Method for *Escherichia coli* and Enterolert Method for Enterococci

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1. SCOPE AND APPLICATION

1.1. This method is used to detect and count *Escherichia coli (E. coli)* and enterococci in non-potable water and is based on Standard Methods SM9223B, EPA Method 1600, and IDEXX Quanti-Tray/2000 Colilert-18 and Enterolert methods. Since *E. coli* and enterococci are commonly found in the intestinal tract of warm-blooded animals, their presence in water samples can indicate the potential presence of fecal pollution and enteric pathogens.

1.2. This procedure is used for samples with Laboratory Information Management System (LIMS) test IDs: ECOLI-18QT, EC-18QT-CL, ENT-24-QT, EN24-QT-CL.

2. SUMMARY OF METHOD

2.1. A 100 mL volume of sample is mixed with the Colilert-18 or Enterolert snap pack media. After mixing, it is poured into a Quanti-Tray 2000 and is run through the heat sealer to seal the tray. The tray is then incubated at 35.0 ± 0.5°C for 18-22 hours for Colilert-18 or at 41 ± 0.5°C for 24-28 hours for Enterolert. For Colilert-18, any wells that are yellow under ambient light and fluorescent under a 365 nm UV light are positive for *E. coli*. For Enterolert, amy wells exhibiting blue fluorescence under a 365 nm UV light are positive for enterococci. The number of positive wells are counted and used to determine the result by referring to the Most Probable Number (MPN) table or using the IDEXX MPN generator software. The MPN value for any diluted samples should be multiplied by the dilution factor to obtain the proper result, unless the dilution has been specified in the IDEXX software.

2.2. Marine water samples and samples with conductivity above 1900 µmho/cm must be diluted at least tenfold with sterile, non-buffered water.

2.3. Interferences

2.3.1. Media is sensitive to prolonged exposure to light. Make sure to store media in the dark. Discard any media that has changed color.

2.4. Definitions

2.4.1. ***Escherichia coli*** – Bacteria that produce a positive total coliform result and possess the enzyme β-glucuronidase, which metabolize the MUG fluorogenic substrate contained in the media, releasing fluorogen, which produces a fluorescent color under a long wave 365 nm UV light.

2.4.2. **Enterococci** – Bacteria that possess the enzyme β-glucosidase, which metabolizes Enterolert's nutrient-indicator, 4-methyl-umberlliferyl β-D-glucoside, and produces a fluorescent color under a long wave 365 nm UV light. This includes *Enterococcus faecalis, E. faecium, E. avium, E. gallinarium* and others from the genus *Enterococcus.*

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3. EQUIPMENT AND SUPPLIES

Record any equipment repairs or maintenance in LIMS\Laboratory Logbook\Maintenance Log

3.1. Sterile gloves, latex or nitrile

3.2. 100 mL sterile, transparent, non-fluorescent IDEXX vessels

3.3. 50 mL disposable beakers

3.4. Chlorine test strips

3.5. Quanti-Tray/2000 trays

3.6. UV light, 365 nm, 6 watt

3.7. Colilert and Colilert-18 Quanti-Tray/2000 comparator

3.8. Quanti-Tray rubber insert for Quanti-Tray/2000

3.9. Quanti-Tray Sealer Model Plus (S/N QTP 13162301048)

3.10. Class A Graduated Cylinder or Class A 100 mL Volumetric Flask

3.11. 250 mL Beaker

3.12. Fisher Scientific Conductivity Meter (S/N 140677692), calibrated per the Fisher Scientific Traceable® Bench Conductivity Meter w/RS-232 Output Instructions and SOP TA-06.25

3.13. Isotemp Refrigerator (S/N 0162177801170112), maintained at 1-5 °C

3.14. Heratherm IMH incubator (S/N 41784672), maintained at 35.0 ± 0.5 °C

3.15. Precision Scientific Incubator Cat No 51221093 (S/N 699121205), maintained at 41 ± 0.5 °C

4. REAGENTS AND STANDARDS

4.1. Colilert-18 snap pack media (for the detection of *E. coli*) – This lab is currently using Colilert-18 but Colilert can also be used if that test better fits program needs.

4.2. Enterolert snap pack media (for the detection of enterococci)

4.3. Deionized (DI) water

4.4. Sterile, non-buffered water (1 L)

4.4.1. Autoclave 1 L of DI water in a 2 L flask for 15 minutes at 121°C and 15 psi. Be careful when removing the flask. Contents are very hot and can be superheated. Use autoclave tape to ensure that the autoclave reached the proper temperature.

4.5. Control cultures (See SOP MB-03.1.)

4.5.1. *Escherichia coli* (*E. coli*) ATCC #11775

4.5.2. *Enterococcus faecalis* (*E. faecalis*) ATCC #19433

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4.5.3. *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC #10145

5. CALIBRATION AND STANDARDIZATION

5.1. See the Laboratory Quality Manual.

6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1. See DEP Field SOP FS 1000 for sample collection and preservation.

7. SAMPLE PREPARATION

7.1. See SOP BB-30 for sample custody and labels.

7.2. Turn on heat sealer and let warm up. The amber power light will turn on when sealer is switched on. The green ready light will turn on when the sealer has warmed up. Place the rubber insert onto the input shelf with the large rectangular cutout facing away from the sealer.

7.3. Retrieve 100 mL sterile IDEXX vessels. For each sample, duplicate and blank, there should be one vessel per analysis. Remove the plastic wrap from the vessel. Place a sample label on each vessel.

Note: When these analyses are being run in conjunction with each other, it is very easy to mix up the tests. Keep everything well organized! It helps to denote which test is being run in each tray. Multi-colored permanent markers can be used to circle or highlight the analysis on the label or the tray. Use one colored marker (usually red) for the Colilert-18 (*E. coli*) test and a different color (usually purple) for the Enterolert (enterococci) test.

8. SAMPLE ANALYSIS

8.1. Shake the sample bottle vigorously, approximately 25 times, to uniformly distribute bacteria. Carefully open bottle. Avoid touching the inside of the bottle to prevent contamination of the sample.

8.1.1. If the test ID includes –CL, check the sample for chlorine with a chlorine test strip. Pour a small volume of sample into a small container (such as a Durham tube), just enough to submerge the pads at the end of the strip. Swirl the test strip in the sample for 30 seconds. If the pads remain colorless, then the sample has already been dechlorinated and should be analyzed. If the pads turn purple, chlorine is present. Do not run the sample. It will be reported as "No Result" with a "Y" qualifier for improper preservation. Record the lot number and result of the chlorine test strip in the **Chlorine Residual** column in Prep Worksheet Manager. See SOP BB-30 for instructions on using Prep Worksheet Manager.

8.2. Pour a small portion of sample into a 50 mL disposable beaker. Check the conductivity of the sample (per the Fisher Scientific Traceable® Bench Conductivity Meter w/RS-232 Output Instructions and SOP TA-06.25) and record in the **Lab**

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Salinity column in Prep Worksheet Manager. See SOP BB-30 for instructions on using Prep Worksheet Manager. If the conductivity is greater than 1,900 µmho/cm, the sample must be diluted tenfold with sterile, non-buffered water.

8.3. Carefully fill the IDEXX vessel with sample up to the 100 mL line. Check the sample ID on the sample container and make sure it matches the sample ID on the IDEXX vessel.

8.3.1. If the sample needs to be diluted, use either a Class A graduated cylinder or glass serological pipette to measure the desired amount of sample into the IDEXX vessel. Bring the volume up to the 100 mL line on the IDEXX vessel with sterile, non-buffered water. Record dilutions in the **Sample Comments 2** column in Prep Worksheet Manager

8.4. Gently tap the media snap pack to ensure that the powder settles to the bottom and is not in the lid of the pack. Open the pack by snapping the top at the score line. Hold the snap pack opening away from face to avoid any residue that comes out when opened. Add the appropriate reagent powder to the sample in the vessel, as indicated by the test ID (Colilert-18 for ECOLI-18QT or EC-18QT-CL and Enterolert for ENT-24-QT or EN24-QT-CL). Cap the vessel and shake vigorously until the powder is dissolved. Record the date and time the reagent powder was added to the first sample in the **Prep Start Date/Time** entry space in Prep Worksheet Manager

8.5. Remove the sample label from the vessel and place it on the well side of the Quanti-Tray. Unthread the lid of the vessel. Hold the top of the Quanti-Tray in one hand with the wells facing the palm. Squeeze the upper part of the tray, so the wells curve toward the palm and carefully pull the foil tab to open the tray. Avoid touching the inside of the tray. Pour the sample/media mixture into the tray. Avoid pouring any sample onto the foil tab. Gently tap the small wells a few times to release any trapped air.

8.6. Place the sample into the rubber insert with the well side down so that the wells fit correctly into the holes of the rubber insert. Carefully slide the insert with the tray into the sealer with the large rectangular cutout on the insert facing away from the sealer until the motor begins to draw it into the sealer. When the tray has completely gone through the sealer (a part of the tray will remain inside, but the internal rollers will no longer be heard), remove the rubber insert and tray from the sealer. Remove the tray from the rubber insert and place the rubber insert back on the input shelf with the large rectangular cutout facing away from the sealer.

8.6.1. If the rubber insert is left in the back of the heat sealer for an extended period of time, the portion that remained in contact with the sealer will be very hot. Handle cautiously to avoid burns.

8.7. Repeat Steps 8.1.-8.6 until all trays for all samples are sealed. Alternately, all samples can be placed in vessels (with sample labels) and mixed with media first. Then, all trays can be filled and sealed.

8.8. When all samples have been sealed, run a blank for each test being analyzed. Fill a sterile Quanti-Tray vessel to the 100 mL line with sterile, non-buffered water. Add the appropriate media to the water and proceed as if it were an actual sample.

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8.8.1. If samples arrive 30 minutes or more after the last Quanti-Tray was processed, run another blank for the test being analyzed.

8.9. Incubate the *E. coli* trays, well side down, at 35 ± 0.5 °C for 18-22 hours. Incubate the enterococci trays, well side down, at 41 ± 0.5 °C for 24-28 hours. Trays can be stacked in the incubator if air flow is not obstructed. Write the incubation date and time on the top tray.

8.10. After incubation, remove the trays and count all positive wells. For *E. coli* trays, count all wells that are yellow and fluorescent equal to or greater than the comparator. Yellow wells should be checked under ambient light. For enterococci, count all wells that exhibit blue fluorescence. When, examining tray for fluorescence, turn off overhead lights and use a 365 nm UV light within 5 inches of the sample to count all wells that are fluorescent. On the well side of the tray, write the number of positive large wells near the large wells at the top of the tray and the number of positive small wells near the small wells at the bottom of the tray.

8.10.1. If any wells are threshold positive, trays may be placed back into the incubator and removed at the end of the incubation window. This will allow the bacteria more time to metabolize the substrate and produce a stronger positive signal.

9. DATA ARCHIVAL

9.1. Record prep, incubation, and read dates/times for each sample on the prep worksheet. Media lot numbers should be entered in their entry spaces, and the sterile non-buffered water lot number should be entered in the **Prep Comments** field. Also, include any pertinent comments and notes, including chlorine checks. Label the duplicated samples with an "**_dup**" after the sample number and duplicate their rows

9.2. Open \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\Micro\QuantiTray Data and navigate to the current year's folder. If one has not already been created, create a new folder saved as the current month (ex. 01 JAN).

9.3. Enter well counts in IDEXX MPN Generator. (To install the MPN Generator, go to the IDEXX website. Under IDEXX Businesses, click on **Water Testing Solutions** and go to **Products and Services**. Under Resources & Support, click on **MPN Generator Software**. Under Resources, click on **MPN Generator Software Program**.)

9.3.1. Open the MPN Generator. Open the appropriate Month/Year folder in \\fldep1\dear\Labs\Lab5\Biology\Bench Common\Micro\QuantiTray Data Highlight and copy the address in the address bar. In the MPN Generator, select **Options…**. In the space labeled **default directory to save files:**, paste the address copied from \\fldep1\...\Lab5... Select the **Date Format: MM/DD/YYYY** and the **Decimal Format: 0.00**. Check the box next to **Use Dilution Mode**. Save changes.

9.3.2. In the blank space at the top of the main MPN Generator page, enter the **File Name** in the format mmddyy-QT, where "mmddyy" is the date the samples were processed. In the **Sample Date** box, enter the analysis date. Fill in the

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Analyst (using LIMS ID), **Method** (Colilert-18 or Enterolert), and **Analyte** (*E. coli* or Enterococci). Enter the volume of undiluted sample as **100**.

9.3.3. Enter the **Sample ID**, the number of **Quanti-Tray®/2000 Positive Large Wells (0-49)** and the number of **Quanti-Tray®/2000 Positive Small Wells (0-48)**. Do not enter anything in the box labeled Quanti-Tray® Positive Wells (0-51). Click **Calculate** and then **Log**.

9.3.3.1. If a sample was diluted, change the volume of undiluted sample before calculating.

9.3.4. Repeat Step 9.3.3. until all the Quanti-Trays have been entered for that date. Change the File Name and Sample Date to reflect a new date being entered.

9.3.4.1. The MPN Generator creates an Excel file in the folder selected in Step 9.3.1. The name of the Excel file is the same as the File Name entered in the MPN Generator; i.e., the date the samples were processed.

9.4. Print the Excel file created by the MPN Generator.

9.4.1. Open the Excel file. Delete Columns F, I, J and K. In Print Preview, select Page Setup. Change the Orientation to **Landscape**. In the Header/Footer tab, select **Custom Header….** In the Center section, type in the File Name. In the Sheet tab, select **Gridlines**. Press OK and Print. Do not save changes.

9.5. Perform a two-person verification on the Excel printout. (Refer to SOP BG-01.)

9.6. Upload results to QC Manager per SOP BB-31.

9.7. Refer to SOP BB-32 for data authorization.

10. QUALITY CONTROL

10.1. Sample Replicates

10.1.1. Analyze at least one laboratory replicate for every 10 samples per analysis per day.

10.1.2. Maintain a control chart of duplicate results. Convert results to logarithms and calculate the range (difference of logarithms of a replicate set). The range should be < 3.27 times the mean range of the last 15 replicate sets.

10.1.3. Corrective Actions: Report laboratory replicate results that do not meet the acceptance criteria with a “J” qualifier.

10.2. Method Blanks

10.2.1. Add 100 mL of sterile, non-buffered water to a mixing vessel and add the appropriate media (Colilert-18 for *E. coli* and Enterolert for enterococci). Shake until dissolved, add to a Quanti-Tray, and seal. Label the tray as a blank and include the test ID and date/time. If more than 30 minutes elapses since the last batch of Quanti-Tray was processed, run a new blank. Incubate blanks with samples for 18-22 hours at 35 ± 0.5 °C for Colilert -18 media or 24 - 28 hours at 41.0 ± 0.5 °C for Enterolert media

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10.2.2. Absence of growth in method blanks demonstrates that media, mixing vessels, Quanti-Trays, and sterile non-buffered water are free of contamination of the target organism from improper handling, inadequate sterilization, or environmental exposure.

10.2.3. Corrective Actions: If method blanks have growth, determine the cause of contamination. The media, mixing vessels, Quanti-Trays, and sterile non-buffered water batch can be checked for sterility. If the cause of contamination has also affected samples, report samples in the batch that are greater than MDL with a "V" qualifier and the comment "Result is V-qualified due to control blank failure."

10.3. Negative Controls

10.3.1. Label a sterile vessel with the media lot number and fill with 100 mL of sterile, non-buffered water. Transfer a small amount of *P. aeruginosa* to the vessel and shake vigorously. Add a media snap pack from the corresponding lot number and mix. Add the control sample to a Quanti-Tray labeled with the lot number, the species, culture serial number, and "N" for negative control and heat seal. Incubate for 18-22 hours at 35 ± 0.5 °C for Colilert -18 media or 24 - 28 hours at 41.0 ± 0.5 °C for Enterolert media.

10.3.2. To check viability of negative control, also add a small amount of the suspension to a BHI broth tube and incubate at 35 ± 0.5 °C for 24 ± 2 hours. If negative control does not grow in BHI broth, then the *P. aeruginosa* may not be viable and the negative control test on the media should be repeated with a new culture.

10.3.3. All wells on the Quanti-Tray/2000 should be negative.

10.3.4. Corrective Actions: If there is any positive well on the negative control tray, repeat the test. If the repeat test is positive, do not use the lot for analysis, and contact supervisor.

10.3.5. Record results in LIMS Standard Preparation Tracker per SOP MB-01.12.

10.4. Positive Controls

10.4.1. For each lot of media, transfer a small amount of *E. coli* for Colilert-18 media or *E. faecalis* for Enterolert media to a sterile vessel containing 100 mL of sterile, non-buffered water and labeled with the species and culture serial number. Add the appropriate media snap pack to the vessel and mix. Add the control sample to a Quanti-Tray labeled with the lot number, the species, culture serial number, and "P" for positive control and heat seal. Incubate *E. coli* at 35 ± 0.5 °C for 18-22 hours for Colilert-18 media and *E. faecalis* at 41.0 ± 0.5 °C for 24-28 hours for Enterolert media.

10.4.2. All wells on the "P" trays should be positive. Refer to step 8.10 for how to read wells.

10.4.3. Corrective Actions: If the trays do not show the correct positive result, repeat the test. If the repeat test is not producing the expected result, do not use the lot for analysis, and contact supervisor.

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10.4.4. Record results in LIMS Standard Preparation Tracker per SOP MB-01.12.

10.5. Volume Check

10.5.1. For each lot of Quanti-Tray mixing vessels, perform a volume check on one vessel. Fill a Class A graduated cylinder or a Class A volumetric flask with 100 mL of DI water. Pour the water into the vessel. If the meniscus reaches the 100 mL line, the lot passes. If the meniscus falls short of the line or goes over it, the lot fails and the check should be redone. If the volume check fails again, do not use the lot. Notify the supervisor. Record results in LIMS Laboratory/Technical/Laboratory Logbook *Pipet-Fun_Cal_Log.*

10.6. Sterility Checks

10.6.1. Check sterility on each lot of sample containers, mixing vessels, and trays with TSB 2X (see SOP MB-02.33). If there is growth (turbidity), recheck lot or batch. If recheck also fails (shows growth), do not use lot and contact supervisor. Record results in LIMS Laboratory/Technical/Laboratory Logbook *Lab_Supplies_QC.*

10.6.1.1. Sample containers and mixing vessels: Add a small amount of TSB 2X (10-20 mL) to the container to be checked. Cap and swirl/shake the media so that it touches every surface area in the container. Incubate at 35 ± 0.5 °C for 24 hours.

10.6.1.2. Trays: In a sterile 250 mL beaker, combine 50 mL TSB 2X with 50 mL sterile, non-buffered water. Pour the mix into the tray and seal. The tray should be full and all surface areas should have had contact with the media. Incubate at 35 ± 0.5 °C for 48 hours.

10.7. Sealer Performance Check

10.7.1. Once a month, add one to two drops of food coloring to 100 mL of DI water. Add mixture to a tray and seal. Check each well for leaks. If leaks are found, repeat the check. If repeat check also fails, do not use the sealer and contact a supervisor. Record results in LIMS Laboratory/Technical/Laboratory Logbook *Instrument_Log.*

10.8. Sodium Thiosulfate Dechlorination Check

10.8.1. Check each lot of sample containers that have a sodium thiosulfate tablet for adequate dechlorination.

10.8.1.1. Dilute bleach to 0.1%. Check the concentration with the Hach Colorimeter II per SOP TA-06.22 and record in Standard Preparation Tracker.

10.8.1.2. Dilute the 0.1% solution to 16 mg/L Cl_2 (also check the concentration with the Colorimeter II) and record the new solution in Standard Preparation Tracker.

10.8.1.3. Add 250 mL of the 16 mg/L Cl_2 solution to the sample container with the tablet that is being tested. Shake until the tablet is dissolved. Use a chlorine test strip to determine if the solution has

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been dechlorinated. If the bleach solution is not dechlorinated, the lot fails, and the check should be redone. If the dechlorination check fails again, do not use the lot. Notify supervisor. Record the date, analyst, SN of the 16 mg/L Cl_2 solution and its concentration reading from the Colorimeter II, the final concentration of the solution after dechlorinating, and the lot number of the chlorine test strips in the ENTRY_COMMENTS of the corresponding sterility check record for the sample container lot in LIMS Laboratory/Technical/Laboratory Logbook *Lab_Supplies_QC*.

11. MAINTENANCE OF QUANTI-TRAY SEALER

11.1. Clean the Quanti-Tray Sealer at least monthly or more often as needed. Refer to the Quanti-Tray PLUS User Manual pdf file located in \\fldep1\DEAR\Labs\Lab5\Biology\Bench Common\Micro\QuantiTray Data for instructions.

12. SAFETY/HAZARDOUS WASTE MANAGEMENT

12.1. All media/bacteria must be autoclaved for 45 minutes before disposal.

12.2. Refer to the Laboratory Health and Safety Plan.

12.3. Read MSDS information for reagent handling and disposal.

13. REFERENCES

13.1. Standard Methods 9223 B-2004

13.2. Standard Methods 9222 B-1997

13.3. Standard Methods 9020-1997

13.4. EPA Method 1600

13.5. IDEXX Quanti-Tray/2000 Colilert-18 method instructions

13.6. IDEXX Quanti-Tray/2000 Enterolert method instructions

13.7. IDEXX Quanti-Tray Sealer PLUS Manual

13.8. NELAC Quality Systems Manual

13.9. FDEP Laboratory Quality Manual http://www.dep.state.fl.us/labs/library/lab_sops.htm

13.10. FDEP Laboratory Health and Safety Plan http://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

13.11. DEP SOP-001/01 FS 1000, *General Sampling Procedures*

13.12. DEP SOP MB-01.5, *Pour Plate Method for Heterotrophic Plate Count*

13.13. DEP SOP MB-02.33, *Preparation of Dehydrated Culture Media*

13.14. DEP SOP MB-03.1, *Maintenance of Bacterial Cultures*

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13.15. DEP SOP BB-30, *Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples*

13.16. DEP SOP BB-31, *LIMS QC Manager Data Uploading Instructions for Bench Biology and Microbiology Samples*

13.17. DEP SOP BB-32, *Test Level Authorization of Uploaded Results for Bench Biology Samples*

APPENDIX OF CHANGES

01/16/2015	Combined Colilert and Enterolert analyses; added chlorine check and analysis blanks; updated Data Archival to reflect the use of the IDEXX MPN Generator; under Quality Control, added volume and sterility checks; added a Maintenance Section; added Quanti-Tray/2000 manuals to References section.
02/26/2015	Changed all tlhlab5 and tlhlab3 network server locations to fldep1 network.
03/16/2017	Updated equipment information, clarified counting procedures, added comparator to equipment list and instructions for use, added instructions for threshold positive wells, added method blanks section to Section 10, and updated updated hyperlinks.

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